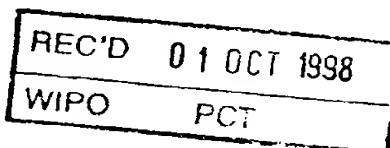


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*Asa Dahlberg*  
Asa Dahlberg

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NEW RECEPTORS

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FIELD OF THE INVENTION

5 The present invention relates to novel vitamin D receptor related (VDRR) polypeptides. Nucleic acid sequences encoding the same, expression vectors containing such sequences and host cells transformed with such expression vectors are also disclosed, as are methods for the expression of the novel VDRR polypeptides of the invention, and uses thereof.

BACKGROUND OF THE INVENTION

15 Nuclear hormone receptors is a large group of conditionally regulated transcription factors. These receptors are activated and regulate target gene expression in response to binding a variety of small chemical molecules (ligands) including steroids, vitamin D3, retinoids, eicosanoids (prostanoids), thyroid hormone and cholesterol derivatives.

20 A growing number of structurally related receptors have been identified for which no ligands yet have been identified. This group of receptors is referred to as orphan nuclear receptors (ONRs). A review of the ONRs can be found in Enmark et al, Mol. Endo., vol. 10, No. 11 (1996) pp. 1293-1307, which is hereby incorporated by reference. The pivotal importance of a number of ONRs for processes such as metabolic homeostasis, cell differentiation and development have been demonstrated both by biochemical and genetic techniques. In addition, several ONRs have also been implicated as key factors in a variety of common diseases and disorders such as diabetes, obesity, inflammatory conditions and proliferative diseases.

25 Based on these findings it is generally believed that novel ONRs are going to become potential drug targets for therapeutic invention of common diseases. Thus, it is of great importance to identify such receptors.

### SUMMARY OF THE INVENTION

The present invention relates to novel vitamin D receptor related (VDRR) polypeptides, and formulations containing the same. Nucleic acid sequences encoding the VDRR polypeptides, expression vectors containing such sequences and host cells transformed with such expression vectors are also disclosed, as are methods for the expression of the novel VDRR polypeptides of the invention. The invention further relates to VDRR polypeptides for use as medicaments, and use of substances affecting VDRR signal transduction for the manufacture of medicaments for treating metabolic, proliferative or inflammatory conditions. The present invention also relates to methods for identifying clones encoding a VDRR polypeptide, methods for identifying ligands to a VDRR and methods for identifying substances for treatment of conditions affected by a VDRR polypeptide. More specifically, the novel VDRR polypeptide can be the polypeptide designated VDRR $\gamma$ , which may be regulated by any small chemical molecule similar in structure to known ligands for nuclear receptors.

### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 - The cDNA sequence encoding the novel nuclear receptor polypeptide vitamin D receptor related gamma (VDRR $\gamma$ ) is shown.

Figure 2 - Evolutionary neighbor-joining tree for VDRR $\gamma$  as given by DBD-HMM alignment.

Figure 3 - Evolutionary neighbor-joining tree for VDRR $\gamma$  as given by LBD-HMM alignment.

Figure 4 - The deduced amino acid sequence of VDRR $\gamma$  is shown.

Figure 5 - Expression of VDRR $\gamma$  in adult human tissues. The numbers on the right hand side, refer to kilobasepairs of the mRNA.

Figure 6 - Vitamin D3 transactivate a GAL4-DBD/VDR-LBD fusion protein but not a GAL4-DBD/VDRR $\gamma$ -LBD fusion protein in transient transfections of CV-1 cells. The number on the left hand side refer to relative luciferase activity of the GAL4-luciferase reporter gene.

Figure 7 - The cDNA sequence encoding VDRR $\gamma$ -2 with an alternatively spliced 5'-end compared to VDRR $\gamma$  is shown.

Figure 8 - The deduced amino acid sequence of VDRR $\gamma$ -2 is shown.

Figure 9 - Heterodimerization of VDRR $\gamma$  with a retinoid X receptor (RXR) is shown.

5 Figure 10 - The effect of pregnenolone derivatives as activators of VDRR $\gamma$  are shown.

Figure 11 - The effect of pregnenolone 16 $\alpha$ -carbonitrile (PCN), dexamethasone and an antiprogesterin (RU486) as activators of VDRR $\gamma$  are shown.

10

### DETAILED DESCRIPTION OF THE INVENTION

The objects above are met by the present invention, which relates to an isolated or recombinant nucleic acid comprising a contiguous nucleic acid sequence encoding a vitamin D receptor related (VDRR) polypeptide. The VDRR polypeptide is suitably of mammalian, preferably human, origin.

15

In preferred embodiments of the present invention, the nucleic acid encoding the VDRR polypeptide contains a DNA-binding domain (DBD) comprising about 77 amino acids with 9 cysteine residues. The DBD is further characterized by the following amino acid sequence identity relative to the DBDs of human Vitamin D Receptor (hVDR) and Orphan Nuclear Receptor 1 isolated from *Xenopus laevis* (xONR1), respectively:

20

(i) at least about 50% amino acid sequence identity with the DBD of hVDR, suitably at least 60% amino acid sequence identity with the DBD of hVDR; and

(ii) at least about 55% amino acid sequence identity with the DBD of xONR1, suitably at least 65% amino acid sequence identity with the DBD of xONR1.

25

More particularly, the amino acid sequence identity relative to the DBDs of hVDR and xONR1, respectively is

(i) about 65% amino acid sequence identity with the DBD of hVDR; and

(ii) about 71% amino acid sequence identity with the DBD of xONR1.

30

In preferred embodiments of the present invention, the nucleic acid encoding the VDRR polypeptide contains a ligand-binding domain (LBD) characterized by the following amino acid sequence identity, relative to the LBDs of hVDR and xONR1, respectively:

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- (i) at least about 30% amino acid sequence identity with the LBD of hVDR, suitably at least 35% amino acid sequence identity with the LBD of hVDR; and
- (ii) at least about 40% amino acid sequence identity with the LBD of xONR1, suitably at least 45% amino acid sequence identity with the LBD of xONR1.

5 More particularly, the amino acid sequence identity relative to the LBDs of hVDR and xONR1, respectively is

- (i) about 42% amino acid sequence identity with the LBD of hVDR; and
- (ii) about 54% amino acid sequence identity with the LBD of xONR1.

10 In particularly preferred embodiments, the nucleic acid sequences of the present invention are those given in Fig. 1 or Fig. 7.

The present invention also relates to a nucleic acid probe for the detection of a nucleic acid sequence encoding a VDRR polypeptide in a sample. Suitably, the probe comprises at least 14 contiguous nucleotides, and preferably at least 28 contiguous nucleotides, of the nucleic acid sequences given in Fig. 1 or Fig. 7. The nucleic acid probe can be used in a method for identifying clones encoding a VDRR polypeptide, wherein the method comprises screening a genomic or cDNA library with the probe under low stringency hybridization conditions, and identifying those clones which display a substantial degree of hybridization to said probe.

20 The present invention further relates to an isolated or recombinant VDRR polypeptide. The polypeptide can be full-length, at which the sequence of amino acids is identical to the corresponding sequence found in mammals in general, and in human beings in particular. In the present invention, the polypeptide can also be a truncated, extended or mutated form of the full-length polypeptide. Truncated and extended forms relate to VDRR polypeptides where one or more amino acids are missing or have been added, respectively, at the N terminal end of the polypeptide chain. Mutated forms relate to VDRR polypeptides where one or more amino acid has been substituted by another amino acid. Suitably, the isolated or recombinant VDRR polypeptide exhibits the amino acid sequences given in Fig. 4 or Fig. 8.

30 The N-terminal sequence of the present nucleic acids encoding VDRR polypeptides, as well as the amino acid sequence of the present VDRR polypeptides, may vary. Thus, various N-terminal isoforms are envisaged, e.g. any of  $\alpha 1$ ,  $\alpha 2$ ,  $\beta 1$ ,  $\beta 2$ ,  $\beta 3$ ,  $\beta 4$ ,  $\gamma 1$  or  $\gamma 2$  as

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disclosed in Fig. 7B of Transcription Factors 3: nuclear receptors, Protein Profile, vol. 2, issue 11 (1995), pp. 1173-1235. This review of nuclear receptors generally is hereby incorporated by reference. More specifically, Vitamin D receptors and related orphans, e.g. ONR1, are discussed at p. 1191-1992.

5 The present invention further relates to pharmaceutical formulations comprising an isolated or recombinant VDRR polypeptide, and one or more therapeutically acceptable excipients. Examples of excipients that can be used are carbohydrates, e.g. monosaccharides, disaccharides and sugar alcohols, such as saccharose and sorbitol. Further examples include amino acids, e.g. histidine and arginine, surfactants, e.g. polyoxyethylene sorbitan fatty acid  
10 esters, inorganic salts, e.g. sodium chloride and calcium chloride, and complexing agents, e.g. EDTA and citric acid.

The present formulation can be in the form of an aqueous solution ready-for-use, or dried, particularly lyophilized. In the latter case, the formulation is reconstituted with a liquid, e.g. sterile water or saline, before use.

15 The present invention further relates to an expression vector comprising an isolated or recombinant nucleic acid, the nucleic acid comprising a contiguous nucleic acid sequence encoding a Vitamin D receptor related (VDRR) polypeptide. The invention also relates to a cell containing such an expression vector.

The present invention further relates to a cell containing an isolated or recombinant  
20 nucleic acid, the nucleic acid comprising a contiguous nucleic acid sequence encoding a Vitamin D receptor related (VDRR) polypeptide.

The present invention further relates to a process for recombinant production of a VDRR polypeptide, by expressing an isolated or recombinant contiguous nucleic acid sequence encoding a Vitamin D receptor related (VDRR) polypeptide in a suitable host cell,  
25 preferably an eukaryotic cell.

The present invention further relates to method for identifying a ligand to a VDRR, e.g. by a cell-based reporter assay, transgenic-animal reporter assay or *in vitro*-binding assay. It also relates to a method for identifying a substance for treatment of a condition affected by a VDRR polypeptide, comprising screening for an agonist or an antagonist of  
30 VDRR polypeptide signal transduction to be used for treating metabolic, proliferative or inflammatory conditions.

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The present invention further relates to a VDRR polypeptide for use as a medicament, as well as use of a substance affecting VDRR signal transduction for the manufacture of a medicament for treating metabolic, proliferative or inflammatory conditions.

More particularly, the present invention can be used for the manufacture of medicaments for treating obesity, diabetes, anorexia, lipoprotein defects, hyperlipidemia, hypercholesteremia or hyperlipoproteinemia. The present invention can be used also for the manufacture of medicaments for treating osteoporosis, rheumatoid arthritis, benign and malignant tumors, hyperproliferative skin disorders or hyperparathyroidism.

The present invention further relates to a method for treating metabolic, proliferative or inflammatory conditions by introducing into a mammal a nucleic acid vector encoding for expression of a VDRR polypeptide. The nucleic acid vector is capable of transforming a cell *in vivo* and expressing said polypeptide in said transformed cell.

The present invention further relates to a method for treatment of a metabolic, proliferative or inflammatory condition by administration of a therapeutically effective amount of a substance affecting VDRR signal transduction, specifically a VDRR polypeptide.

In the present invention, the term "isolated" in connection with VDRR polypeptides or nucleic acids encoding the same, relates to nucleic acids or polypeptides that have been isolated from a natural source, e.g. the liver, small intestine or colon of a human being. The isolated VDRR polypeptides or nucleic acids of the present invention are unique in the sense that they are not found in a pure or separated form in nature. Use of the term "isolated" indicates that a naturally occurring sequence has been removed from its normal cellular environment. Thus, the sequence may be in a cell-free environment or in a different cellular environment. The term does not imply that the sequence is the only nucleic acid or amino acid sequence present, but that it is the predominant nucleic acid or amino acid sequence present. Furthermore, the nucleic acid or polypeptide should be essentially free of non-amino acid or non-nucleic acid material naturally associated with the respective product. In this context, essentially free relates to more than 80%, suitably more than 90%, and preferably more than 95% purity.

The inventors of the present invention, have surprisingly isolated a novel nucleic acid sequence, and a polypeptide encoded by said nucleic acid sequence. Thus, a novel cDNA encoding a polypeptide designated VDRR $\gamma$  has been cloned and characterized. This poly-

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peptide is, based on amino acid sequence similarity, a novel member of the nuclear (hormone) receptor supergene family. Hidden Markov Models (HMMs) in combination with phylogenetic analysis such as neighbor-joining tree methods and other statistical algorithms shows that VDRR $\gamma$  belong to a sub-family of vitamin D receptors (VDRs) and a VDR-like receptor from *Xenopus laevis* designated xONR1 (see Smith et al., Nucl. Acids Res., 22 (1994), No. 1, pp. 66-71). The VDRR $\gamma$ , therefore, is one member of a family of Vitamin D receptor related (VDRR) polypeptides.

This finding, in combination with the highly restricted expression pattern we observe for human VDRR $\gamma$  (liver, small intestine and mucosa of colon) and in analogy to other nuclear receptors exhibiting a tissue specific expression pattern such as the peroxisome proliferator-activated receptors (PPARs) - suggest that VDRR $\gamma$  performs important physiological functions in liver, small intestine and colon. Accordingly, VDRR $\gamma$  is likely to be an important sensor of key metabolic pathways affecting lipid, carbohydrate or amino acid metabolism/homeostasis. In addition, the highly selective tissue specific expression pattern suggest that VDRR $\gamma$  may participate in cellular differentiation and development of these tissues.

An additional human VDRR $\gamma$  cDNA with an alternatively spliced 5'-end has been identified (see Fig. 7). The VDRR $\gamma$  cDNAs are thus able to encode at least one alternative N-terminal variant (Fig. 8) in addition to the VDRR $\gamma$  polypeptide shown in Fig. 4. In analogy to other members of the nuclear receptor supergene family such as ROR $\alpha$  and RAR $\alpha$  these N-terminal isoforms of VDRR $\gamma$  may specify different functions including DNA-binding specificity and/or promoter specific activation (Gronemeyer and Laudet, 1995).

In the present specification, the term VDRR $\gamma$  relates to the various polypeptides corresponding to the differentially spliced VDRR $\gamma$  cDNAs including VDRR $\gamma$ -1 and VDRR $\gamma$ -2. However, when reference is made to Fig. 1 and Fig. 4, VDRR $\gamma$  cDNA and VDRR $\gamma$  relates specifically to VDRR $\gamma$ -1 cDNA and VDRR $\gamma$ -1, respectively. In the same way, when reference is made to Fig. 7 and Fig. 8, VDRR $\gamma$  cDNA and VDRR $\gamma$  relates specifically to VDRR $\gamma$ -2 cDNA and VDRR $\gamma$ -2, respectively.

In contrast to the VDRR $\gamma$ -2 cDNA, the VDRR $\gamma$ -1 cDNA does not contain a classical AUG initiation codon but instead may initiate at an alternative CUG codon. This putative



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non-AUG start site is located in a favorable sequence context for efficient initiation from alternative start sites and is in frame with the entire open reading frame and preceded by a stop codon.

5 Taken together, the VDRRs in general, and more specifically the VDRR $\gamma$ , may be important in

- 1) metabolic diseases such as obesity, diabetes (type I and II), lipoprotein disorders,
- 2) proliferative conditions such as tumors (benign and malignant) of the small intestine and colon,
- 3) ulcero-inflammatory diseases of small intestine and colon such as Crohn's disease
- 10 and ulcerative colitis, and
- 4) congenital anomalies of small intestine and colon.

The high amino acid sequence identity of VDRR $\gamma$  with the VDR both in the DNA-binding domain (DBD) and ligand-binding domain (LBD) indicate that these two receptors may also have overlapping yet distinct functional characteristics. In analogy, retinoic acid

15 receptors (RARs) and retinoid X receptors (RXRs) have similar amino acid sequence identities in the DBD and LBD region as the VDR and VDRR $\gamma$ . RARs and RXRs have been shown to have distinct functional similarities such that both receptors bind 9-cis retinoic acid and have overlapping DNA-binding specificities and accordingly regulate overlapping gene networks. Based on these findings, VDRR $\gamma$  may be regulated by small chemical molecules

20 similar in structure to known ligands for nuclear receptors but not necessarily identical to ligands for the 1 $\alpha$ , 25-dihydroxy vitamin D3 receptor. Furthermore, VDRR $\gamma$  may regulate vitamin D3 responsive gene networks by binding to a Vitamin D responsive element (VDRE)-like DNA sequence. In the present application, the 1 $\alpha$ , 25-dihydroxy vitamin D3 receptor is abbreviated as the Vitamin D receptor (VDR).

25 In the present invention, the substance affecting VDRR signal transduction can be any small chemical molecule of natural or synthetic origin, e.g. a carbohydrate such as an aromatic compound. The small molecule may have a molecular weight in the range of from about 100 up to about 500 Da. Suitably, the small chemical molecule has a molecular weight in the range of from 200 up to 400 Da. Preferably, the small chemical molecule has a

30 molecular weight of about 300 Da.

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The human VDRR $\gamma$  polypeptides, including VDRR $\gamma$ -1 and VDRR $\gamma$ -2, have been shown to be activated e.g. by pregnenolones and estradiol (weakly), but not by certain other steroid hormones such as cortisol, aldosterone, progesterone and estrogen, and most likely not by progestines and glucocorticoids. Thus, human VDRR $\gamma$  is not activated by

- 5 pregnenolone 16 $\alpha$ -carbonitrile (PCN), a glucocorticoid antagonist. For this reason, human VDRR $\gamma$  can also be designated human pregnenolone activated (nuclear) receptors (hPAR). Information about pregnenolone can be found e.g. in the Merck Index, 11th ed., Merck & Co., Inc. Rahway, N.J., USA, p. 7735, 1989.

- 10 Activators for human VDRR $\gamma$  polypeptides, including VDRR $\gamma$ -1 and VDRR $\gamma$ -2, (hPAR-1 and hPAR-2, respectively), include but are not limited to pregnenolones, such as pregnane-ones, pregnane-diones, pregnane-triones, and pregnane-diols, and androstanes, such as androstane-ols, and androstane-diols. Suitably, the pregnenolones are non-planar, particularly 5 $\beta$ -pregnanes.

- 15 Specific examples of activators and possibly ligands for human VDRR $\gamma$  polypeptides, including VDRR $\gamma$ -1 and VDRR $\gamma$ -2, are the following compounds, which are marketed by Sigma-Aldrich of Sweden:

- i) 5 $\beta$ -pregnane-3,20-dione
- ii) 3 $\alpha$ -hydroxy-5 $\beta$ -pregnane-11,20-dione methanesulphonate
- iii) 5 $\beta$ -pregnane-3 $\alpha$ ,20 $\beta$ -diol
- 20 iv) pregnenolone
- v) Pregn-4-eno[16,17- $\delta$ ][2]isoxazolline-3,20-dione, 6 $\alpha$ -methyl-3'-phenyl-, ethyl ether solvate
- vi) Pregna-1,4,9(11)-trien-3,20-dione, 21-[4-[6-methoxy-2-(4-morpholinyl)-4-pyrimidinyl]-1-piperazinyl]-16-methyl-, (16 $\alpha$ )-
- 25 vii) Estran-3-ol, 17-[[[3-(trifluoromethyl)phenyl]methyl]amino]-, (E)-2-butenedioate (1:1) (salt)
- viii) 9 $\alpha$ -Fluoro-5 $\alpha$ -androstane-11 $\beta$ ,17 $\beta$ -diol
- ix) Spiro[5 $\alpha$ -androstane-3,2'-benzothiazolin]-11-one, 17 $\beta$ -hydroxy-17-methyl-
- x) Spiro[pregnane-3,2'-thiazolidine]-4'-carboxylic acid, 11 $\alpha$ -hydroxy-20-oxo-,
- 30 sodium salt
- xi) 17 $\beta$ -Dimethylamino-17-ethynyl-5 $\alpha$ -androstane-11 $\beta$ -ol

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xii) 6 $\beta$ -Hydroxy-3,5-cyclo-5 $\alpha$ -pregnan-20-one, nitritexiii) 3 $\alpha$ -Hydroxy-5 $\beta$ -pregnane-11,20-dione, acetate, 20-O-(methylsulfonyl)-oximexiv) 17 $\alpha$ -Methyl-5 $\alpha$ -androstane-11 $\beta$ ,17-diolxv) 5 $\beta$ -Pregnane-3,11,20-trione, trioxime

- 5 xvi) 3 $\alpha$ -Hydroxy-5 $\beta$ -pregnane-11,20-dione, 20-hydrazone with hydrazide of  
1-(carboxymethyl) pyridinium chloride.

Genes coding for polypeptides, such as human vitamin D receptor related gamma (hVDRR $\gamma$ ), may be cloned by incorporating a DNA fragment coding for the polypeptide into a recombinant DNA vehicle, e.g. a vector, and transforming suitable prokaryotic or eukaryotic host cells. Such recombinant DNA techniques are well known and e.g. described in  
10 Methods in Enzymology, Academic Press, San Diego, CA, USA (1994), vols. 65 and 68 (1979), and vols. 100 and 101 (1983).

The host cells for use in the present invention can be prokaryotic or eukaryotic, preferably eukaryotic cells. Suitable eukaryotic host cells include but are not limited to cells  
15 from yeast, e.g. Saccharomyces, insect cells and mammalian cells such as Chinese Hamster Ovary (CHO), Baby Hamster Kidney (BHK), COS and the like. Suitable prokaryotic host cells include but are not limited to cells from Enterobacteriaceae, e.g. E. coli, Bacillus and Streptomyces.

20

### EXAMPLES

The following Examples are provided for purposes of illustration only and are not to be construed as in any way limiting the scope of the present invention, which is defined by the appended claims.

25

#### EXAMPLE 1

##### Identification and isolation of human VDRR $\gamma$ cDNA

Expressed Sequence Tag (EST) databases were screened for nuclear receptor related sequences with a DNA-binding domain (DBD) profile of nuclear receptors. This search  
30 profile was created by multiple alignment of a selected set of nuclear receptor sub-domains followed by a statistical calculation to obtain a so called Hidden Markov Model (HMM) of

different subfamily members of the nuclear receptor supergene family. The cDNA of one of the nuclear receptor related EST sequences identified (Incyte clone no 2211526) was analyzed in detail by sequencing. After DNA sequencing of the entire Incyte cDNA clone (approximately 2200 basepairs) the clone was found to encode a putative ligand-binding domain (LBD) with 54% and 44% similarity to xONR-1 and to the vitamin D receptor (VDR), respectively. The cDNA of the Incyte clone was not full-length and did not encode a sequence corresponding to a complete DBD.

5'-RACE (rapid amplification of cDNA ends) of random primed cDNA from human liver RNA (Invitrogen) followed by cloning and DNA sequencing showed that the 5'-part of the cDNA corresponding to the Incyte clone encoded a DBD characteristic for nuclear receptors and with 71% and 65% sequence identity to xONR-1 and VDR, respectively. Multiple alignments in combination with evolutionary neighbor-joining tree analysis placed the polypeptide encoded by the cDNA (specified in Fig. 1) in the group of VDRs (Figs. 2 and 3) and was named human vitamin D receptor related gamma (VDRR $\gamma$ ). The deduced amino acid sequence of VDRR $\gamma$  is given in Fig. 4.

## EXAMPLE 2

### Expression of VDRR $\gamma$ mRNA in human tissues

Multiple tissue northern blots (Clontech) was used to determine the expression pattern of VDRR $\gamma$  in adult human tissues. As shown in Fig. 5, VDRR $\gamma$  is abundantly expressed in small intestine, mucosal lining of colon and liver but not in several other tissues including spleen, thymus, prostate, testis, ovary, peripheral blood leukocytes, heart, brain, placenta, lung, skeletal muscle, kidney and pancreas. To investigate if VDRR $\gamma$  was expressed at lower levels in any of the other tissues examined, the filter was exposed for an extended time (one week as compared to overnight). Even after this prolonged exposure (data not shown), expression could still only be detected in the same tissues and not in any of the other tissues examined. The restricted expression pattern of VDRR $\gamma$  suggest that this receptor is likely to have an important regulatory function in liver and intestine.

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EXAMPLE 3Transient transfections of GAL4-DBD/VDRRy-LBD fusion protein using Vitamin D3

Transient transfections were performed to analyze if vitamin D3 activate the VDRRy polypeptide. To this end, transient co-transfections of CV-1 cells were performed with expression plasmids encoding fusion proteins of the GAL4-DBD fused to the LBD of either the VDR or the VDRR together with a reporter-plasmid containing five GAL4 responsive elements upstream of the luciferase gene. After transfection, cells were treated with vehicle (DMSO) alone or with vitamin D3 for 48 hours followed by harvesting of the cells and measurement of the luciferase activity in cell extracts. As shown in Fig. 6, vitamin D3 (1 µM) transactivate the GAL4-DBD/VDR-LBD but not the corresponding GAL4-DBD/-VDRRy-LBD polypeptide under these conditions. This indicates that the two receptors may have distinct ligand-binding specificities.

15

EXAMPLE 4Identification and isolation of human VDRRy cDNAs encoding multiple N-terminal isoforms

5'-RACE (see Example 1) of cDNA from human liver RNA followed by cloning and DNA sequencing identified an additional human VDRRy cDNA with alternatively spliced 5'-end (see Fig. 7). The VDRRy cDNAs are thus able to encode at least one alternative N-terminal variant (Fig. 8) in addition to the VDRRy polypeptide shown in Fig. 4. The polypeptides disclosed in Fig. 4 and Fig. 8 which correspond to the differentially spliced VDRRy cDNAs are designated as VDRRy-1 and VDRRy-2, respectively.

EXAMPLE 5VDRRy heterodimerise with RXR and bind to direct repeats (DRs) spaced by three or four nucleotides

- 5 Expression plasmids containing VDRRy or RXR $\beta$  cDNAs were transcribed using T7 polymerase and translated *in vitro* in TNT reticulocyte lysates (Promega, Madison, WI, USA). To investigate the DNA-binding specificity of VDRRy a native gel mobility assay was employed essentially as described (Berkenstam et al., Cell, 69, 401-412, 1992) in which *in vitro* translated VDRRy was incubated in the presence or absence of *in vitro* translated
- 10 RXR $\beta$  with different 32P-labelled direct repeats (DR-1 to DR-5) as indicated in Fig. 9. The direct repeats were derived from the DR-5 element in the RAR- $\beta$ 2 promoter (de Thé et al., Nature, 343, 177-180, 1990) and modified to be separated by one to five nucleotides (Pettersson et al., Mechanisms of Dev., 54, 1-13, 1995). Protein-DNA complexes were separated on native 5% polyacryl-amide/0.25xTBE gels followed by autoradiography. As
- 15 shown in Fig. 9, of the five DRs tested efficient VDRRy binding could only be detected with DRs separated by three or four nucleotides and only in the presence of RXR. However, weaker RXR-dependent binding could also be observed to DR-2 and DR-1 elements. These results demonstrate that VDRRy require RXR heterodimerisation for efficient DNA-binding to a specific subset of DRs. These results, however, do not exclude the possibility that
- 20 VDRRy may bind as a monomer, dimer or heterodimer to distinct but related DNA-sequences. Importantly, our results demonstrate that VDRRy and other nuclear receptors including the VDR (e.g. Markose, E. R. et al., Proc. Natl. Acad. Sci. USA, 87, 1701-1705, 1990), THR $\alpha$ s (e.g. Gronemeyer, H. and Moras, D., Nature, 375, 190-191, 1995), LXR $\alpha$ s (e.g. Willy, P. J. et al., Genes. Dev., 9, 1033-1045, 1995), share common target DNA-sequence
- 25 specificity and thus may regulate overlapping gene networks.

EXAMPLE 6Pregnenolone derivatives as activators of VDRR $\gamma$ 

For identifying activators or ligands for VDRR $\gamma$ , a library of substances structurally  
5 biased towards different classes of activators and ligands for nuclear receptors were tested.  
The activation of VDRR $\gamma$  was analyzed in a reporter gene assay in transiently Caco-2 (TC7)  
cells (Carriere et al, 1994). In this initial screen, the synthetic substances with ability to  
activate VDRR $\gamma$  were found to be structurally similar to pregnenolones (data not shown).  
Based on these results, naturally occurring pregnenolone derivatives were examined for  
10 activation of VDRR $\gamma$ . The results are shown in Fig. 10. As is evident from Fig. 10, VDRR $\gamma$   
was activated about 5 to 12 fold by pregnenolone, 5 $\beta$ -pregnane-3,20-dione, 5 $\beta$ -pregnane-  
3 $\alpha$ ,20 $\beta$ -diol and 3 $\alpha$ -hydroxy-5 $\beta$ -pregnane-11,20-dione methanesulphonate. In contrast to  
the efficient activation observed by the 5 $\beta$ -pregnane-3,20-dione, the corresponding planar  
steroid derivative 5 $\alpha$ -pregnane-3,20-dione did not activate the receptor. Other 5 $\beta$ -pregnanes  
15 also activated VDRR $\gamma$  efficiently as opposed to all planar pregnenolone derivatives tested, as  
is also evident from Fig. 10.

EXAMPLE 7

20 Pregnenolone 16 $\alpha$ -carbonitrile (PCN), dexamethasone and an antiprogesterin (RU486) as  
activators of VDRR $\gamma$

Further experiments were performed to find out if pregnenolone 16 $\alpha$ -carbonitrile  
(PCN), a glucocorticoid antagonist or dexamethasone are activators of VDRR $\gamma$ . To this  
effect, Caco-2 cells were transfected as before with VDRR $\gamma$  and the activation was analyzed  
25 after treatment of the cells with 10  $\mu$ M PCN or dexamethasone. The results are shown in  
Fig. 11. As is evident from Fig. 11, VDRR $\gamma$  was not activated by these substances, indicat-  
ing that VDRR $\gamma$  is not the human PCN receptor. This suggestion is corroborated by the  
observation that also the antiprogesterin RU486 only caused a slight increase (two fold) in  
VDRR $\gamma$  mediated reporter gene activity as is evident from Fig. 11.

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CLAIMS

1. An isolated or recombinant nucleic acid comprising a contiguous nucleic acid sequence  
5 encoding a vitamin D receptor related (VDRR) polypeptide.
2. The nucleic acid according to claim 1, wherein said VDRR polypeptide is of mammalian, preferably human, origin.
- 10 3. The nucleic acid according to claims 1 or 2, encoding the VDRR polypeptide containing a DNA-binding domain (DBD) comprising about 77 amino acids with 9 cysteine residues, wherein said DBD is further characterized by the following amino acid sequence identity relative to the DBDs of human Vitamin D Receptor (hVDR) and Orphan Nuclear Receptor 1 isolated from *Xenopus laevis* (xONR1), respectively:  
15 (i) at least about 50% amino acid sequence identity with the DBD of hVDR; and  
(ii) at least about 55% amino acid sequence identity with the DBD of xONR1.
4. The nucleic acid according to claim 3, wherein said DBD is characterized by the following amino acid sequence identity:  
20 (i) at least 60% amino acid sequence identity with the DBD of hVDR; and  
(ii) at least 65% amino acid sequence identity with the DBD of xONR1.
5. The nucleic acid according to claim 4, wherein said DBD is characterized by the following amino acid sequence identity:  
25 (i) about 65% amino acid sequence identity with the DBD of hVDR; and  
(ii) about 71% amino acid sequence identity with the DBD of xONR1.
6. The nucleic acid according to any previous claim, encoding the VDRR polypeptide, wherein the ligand-binding domain (LBD) of said polypeptide is characterized by the  
30 following amino acid sequence identity, relative to the LBDs of hVDR and xONR1, respectively:



- (i) at least about 30% amino acid sequence identity with the LBD of hVDR; and
- (ii) at least about 40% amino acid sequence identity with the LBD of xONR1.

7. The nucleic acid according to claim 6, wherein said LBD is characterized by the following  
5 amino acid sequence identity:

- (i) at least 35% amino acid sequence identity with the LBD of hVDR; and
- (ii) at least 45% amino acid sequence identity with the LBD of xONR1.

8. The nucleic acid according to claim 7, wherein said LBD is characterized by the following  
10 amino acid sequence identity:

- (i) about 42% amino acid sequence identity with the LBD of hVDR; and
- (ii) about 54% amino acid sequence identity with the LBD of xONR1.

9. The nucleic acid according to any previous claim, wherein said nucleic acid sequence is  
15 that given in Fig. 1 or Fig. 7.

10. A nucleic acid probe for the detection of a nucleic acid sequence encoding a VDRR  
polypeptide in a sample.

20 11. The nucleic acid probe according to claim 10, wherein said probe comprises at least 14  
contiguous nucleotides of the nucleic acid sequence given in Fig. 1 or Fig. 7.

12. A method for identifying clones encoding a VDRR polypeptide said method comprising  
screening a genomic or cDNA library with a nucleic acid probe according to claims 10 or 11  
25 under low stringency hybridization conditions, and identifying those clones which display a  
substantial degree of hybridization to said probe.

13. An expression vector comprising a nucleic acid according to claim 1.

30 14. A cell containing a nucleic acid according to claim 1.

15. A cell containing an expression vector according to claim 13.

16. A process for recombinant production of a VDRR polypeptide, said process comprising expressing the nucleic acid of claim 1 in a suitable host cell.

5

17. The process according to claim 16, wherein the host cell is eukaryotic.

18. An isolated or recombinant VDRR polypeptide.

10 19. The isolated or recombinant VDRR polypeptide according to claim 18 comprising the amino acid sequence given in Fig. 4 or Fig. 8.

20. A pharmaceutical formulation comprising an isolated or recombinant VDRR polypeptide according to claims 18 or 19, and one or more therapeutically acceptable

15 excipients.

21. A method for identifying a ligand to a VDRR, by a cell-based reporter assay, transgenic-animal reporter assay or *in vitro*-binding assay.

20 22. A method for identifying a substance for treatment of a condition affected by a VDRR polypeptide, comprising screening for an agonist or an antagonist of VDRR polypeptide signal transduction to be used for treating metabolic, proliferative or inflammatory conditions.

25 23. A VDRR polypeptide for use as a medicament.

24. Use of a substance affecting VDRR signal transduction for the manufacture of a medicament for treating metabolic, proliferative or inflammatory conditions.

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25. Use of a substance affecting VDRR signal transduction for the manufacture of a medicament for treating obesity, diabetes, anorexia, lipoprotein defects, hyperlipidemia, hypercholesteremia or hyperlipoproteinemia.

- 5 26. Use of a substance affecting VDRR signal transduction for the manufacture of a medicament for treating osteoporosis, rheumatoid arthritis, benign and malign tumors, hyperproliferative skin disorders or hyperparathyroidism.

- 10 27. Use according to any of claims 24-26, wherein the substance affecting VDRR signal transduction is a chemical molecule of natural or synthetic origin with a molecular weight in the range of from about 100 up to about 500 Da, preferably with a molecular weight of about 300 Da.

- 15 28. A method for treating metabolic, proliferative or inflammatory conditions comprising introducing into a mammal a nucleic acid vector according to claim 13 encoding for expression of a VDRR polypeptide and wherein said nucleic acid vector is capable of transforming a cell *in vivo* and expressing said polypeptide in said transformed cell.

- 20 29. A method for treatment of a metabolic, proliferative or inflammatory condition by administration of a therapeutically effective amount of a substance affecting VDRR signal transduction.

- 25 30. The method according to claim 29, wherein the substance affecting VDRR signal transduction is a chemical molecule of natural or synthetic origin with a molecular weight in the range of from about 100 up to about 500 Da, preferably with a molecular weight of about 300 Da.

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ABSTRACT

The present invention relates to novel vitamin D receptor related (VDRR) poly-  
5 peptides, and formulations containing the same. Nucleic acid sequences encoding the  
VDRR polypeptides, expression vectors containing such sequences and host cells trans-  
formed with such expression vectors are also disclosed, as are methods for the expression  
of the novel VDRR polypeptides of the invention. The invention further relates to VDRR  
polypeptides for use as medicaments, and use of substances affecting VDRR signal trans-  
10 duction for the manufacture of medicaments for treating metabolic, proliferative or inflam-  
matory conditions. The present invention also relates to methods for identifying clones  
encoding a VDRR polypeptide, methods for identifying ligands to a VDRR and methods  
for identifying substances for treatment of conditions affected by a VDRR polypeptide.  
More specifically, the novel VDRR polypeptide can be the polypeptide designated  
15 VDRR $\gamma$ , which may be regulated by any small chemical molecule similar in structure to  
known ligands for nuclear receptors.

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1 CCTCTGAAGG TTCTAGAATC GATAGTGAAT TCGTGGGACG GGAAGAGGAA  
51 GCACTGCCTT TACTTCAGTG GGAATCTCGG CCTCAGCCTG CAAGCCAAAGT  
101 GTTCACAGTG AAAAAAGCAA GAGAATAAGC TAATACTCCT GTCCTGAACA  
151 AGGCAGCGGC TCCTTGGTAA AGCTACTCCT TGATCGATCC TTTGCACCGG  
201 ATTGTTCAAA GTGGACCCCA GGGGAGAAGT CGGAGCAAAG AACTTACCAC  
251 CAAGCAGTCC AAGAGGCCCA GAAGCAAACC TGGAGGTGAG ACCCAAAGAA  
301 AGCTGGAACC ATGCTGACTT TGTACACTGT GAGGACACAG AGTCTGTTCC  
351 TGGAAAGCCC AGTGTCACG CAGATGAGGA AGTCGGAGGT CCCCCAATCT  
401 GCCGTGTATG TGGGGACAAG GCCACTGGCT ATCACTTCAA TGTCATGACA  
451 TGTGAAGGAT GCAAGGGCTT TTTCAGGAGG GCCATGAAAC GCAACGCCCCG  
501 GCTGAGGTGC CCCTTCCGGA AGGGCGCCTG CGAGATCACC CGGAAGACCC  
551 GGCGACAGTG CCAGGCCTGC CGCCTGCGCA AGTGCCTGGA GAGCGGCATG  
601 AAGAAGGAGA TGATCATGTC CGACGAGGCC GTGGAGGAGA GCGGGGCCTT  
651 GATCAAGCGG AAGAAAAGTG AACGGACAGG GACTCAGCCA CTGGGAGTGC  
701 AGGGGCTGAC AGAGGAGCAG CGGATGATGA TCAGGGAGCT GATGGACGCT  
751 CAGATGAAAA CCTTTGACAC TACCTTCTCC CATTTCAAGA ATTTCCGGCT  
801 GCCAGGGGTG CTTAGCAGTG GCTGCGAGTT GCCAGAGTCT CTGCAGGCCC  
851 CATCGAGGGA AGAAGCTGCC AAGTGGAGCC AGGTCCGGAA AGATCTGTGC  
901 TCTTTGAAGG TCTCTCTGCA GCTGCGGGGG GAGGATGGCA GTGTCTGGAA  
951 CTACAAACCC CCAGCCGACA GTGGCGGGAA AGAGATCTTC TCCCTGCTGC  
1001 CCCACATGGC TGACATGTCA ACCTACATGT TCAAAGGCAT CATCAGCTTT  
1051 GCCAAAGTCA TCTCCTACTT CAGGGACTTG CCCATCGAGG ACCAGATCTC  
1101 CCTGCTGAAG GGGGCCGCTT TCGAGCTGTG TCAACTGAGA TTCAACACAG  
1151 TGTTCACGC GGAGACTGGA ACCTGGGAGT GTGGCCGGCT GTCCTACTGC  
1201 TTGGAAGACA CTGCAGGTGG CTTCAGCAA CTTCTACTGG AGCCCATGCT  
1251 GAAATTCCAC TACATGCTGA AGAAGCTGCA GCTGCATGAG GAGGAGTATG  
1301 TGCTGATGCA GGCCATCTCC CTCTTCTCCC CAGACCGCCC AGGTGTGCTG  
1351 CAGCACCGCG TGGTGGACCA GCTGCAGGAG CAATTCGCCA TTA CTCTGAA  
1401 GTCCTACATT GAATGCAATC GGCCCCAGCC TGCTCATAGG TTCTTGTTCC

Fig. 1

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1451 TGAAGATCAT GGCTATGCTC ACCGAGCTCC GCAGCATCAA TGCTCAGCAC  
1501 ACCCAGCGGC TGCTGCGCAT CCAGGACATA CACCCCTTTG CTACGCCCTT  
1551 CATGCAGGAG TTGTTGGCA TCACAGGTAG CTGAGCGGCT GCCCTTGGGT  
1601 GACACCTCCG AGAGGCAGCC AGACCCAGAG CCCTCTGAGC CGCCACTCCC  
1651 GGGCCAAGAC AGATGGACAC TGCCAAGAGC CGACAATGCC CTGCTGGCCT  
1701 GTCTCCCTAG GGAATTCCTG CTATGACAGC TGGCTAGCAT TCCTCAGGAA  
1751 GGACATGGGT GGGGGGACC CCCAGTTCAG TCTGTAGGGA GTGAAGCCAC  
1801 AGACTCTTAC GTGGAGAGTG CACTGACCTG TAGGTCAGGA CCATCAGAGA  
1851 GGCAAGGTTG CCCTTTCCTT TTAAGGACC CTGTGGTCTG GGGAGAAATC  
1901 CCTCAGATCC CACTAAAGTG TCAAGGTGTG GAAGGGACCA AGCGACCAAG  
1951 GATAGGCCAT CTGGGGTCTA TGCCACATA CCCACGTTTG TTCGCTTCCT  
2001 GAGTCTTTTC ATTGCTACCT CTAATAGTCC TGTCTCCAC TTCCACTCG  
2051 TTCCCTCCT CTCCGAGCT GCTTTGTGGG CTCAAGGCCT GTACTCATCG  
2101 GCAGGTGCAT GAGTATCTGT GGGAGTCCTC TAGAGAGATG AGAAGCCAGG  
2151 AGGCCTGCAC CAAATGTCAG AAGCTTGGCA TGACCTCAIT CCGGCCACAT  
2201 CATCTGTGT CTCTGCATCC ATTTGAACAC ATTATTAAGC ACTGATAATA  
2251 GGTAGCCTGC TGTGGGTAT ACAGCATTGA CTCAGATATA GATCCTGAGC  
2301 TCACAGAGTT TATAGTTAAA AAAACAAACA GAAACACAAA CAATTTGGAT  
2351 CAAAAGGAGA AAATGATAAG TGACAAAAGC AGCACAAGGA ATTTCCCTGT  
2401 GTGGATGCTG AGCTGTGATG GCAGGCACTG GGTACCCAAG TGAAGGTTC  
2451 CGAGGACATG AGTCTGTAGG AGCAAGGGCA CAACTGCAG CTGTGAGTGC  
2501 GTGTGTGTGA TTTGGTGTAG GTAGGTCTGT TTGCCACTTG ATGGGGCCTG  
2551 GGTGTGTTC TGGGGCTGGA ATGCTGGGTA TGCTCTGTGA CAAGGCTACG  
2601 CTGACAATCA GTTAAACACA CCGGAGAAGA ACCATTTACA TGCACCTTAT  
2651 ATTTCTGTGT ACACATCTAT ICTCAAAGCT AAAGGGTATG AAAGTGCCCTG  
2701 CCTTGTTTAT AGCCACTTGT GAGTAAAAAT TTTTGTGCAT TTTCACAAAT  
2751 TATACTTTAT ATAAGGCATT CCACACCTAA GAAC TAGTTT TGGGAAATGT  
2801 AGCCCTGGGT TTAATGTCAA ATCAAGGCAA AAGGAATTAA ATAATGTACT  
2851 TTTGGCTAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA  
2901 AAAAA

Fig. 1 (cont.)

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## Evolutionary Neighbour-Joining Tree

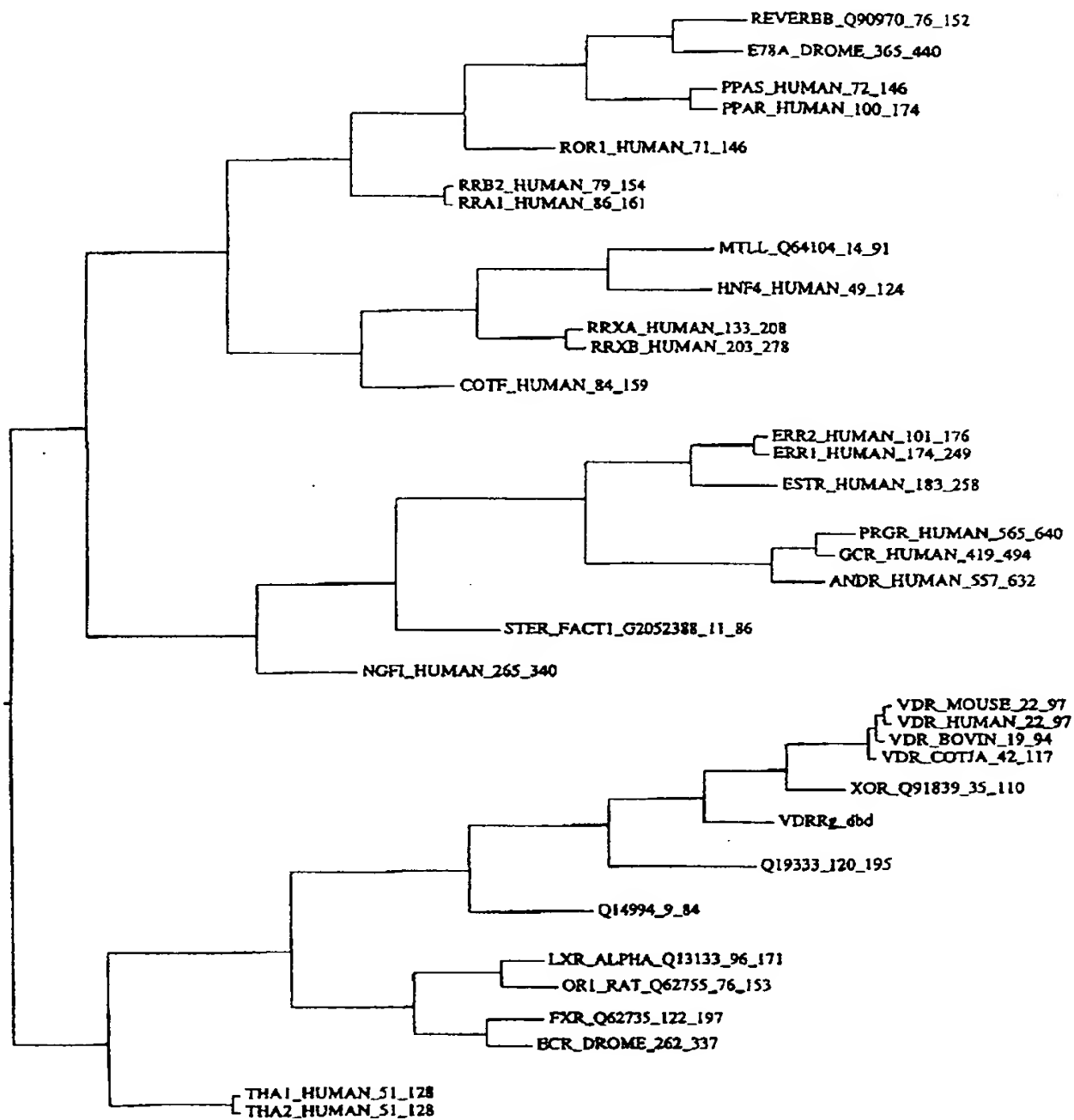


Fig. 2

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## Evolutionary Neighbour-Joining Tree

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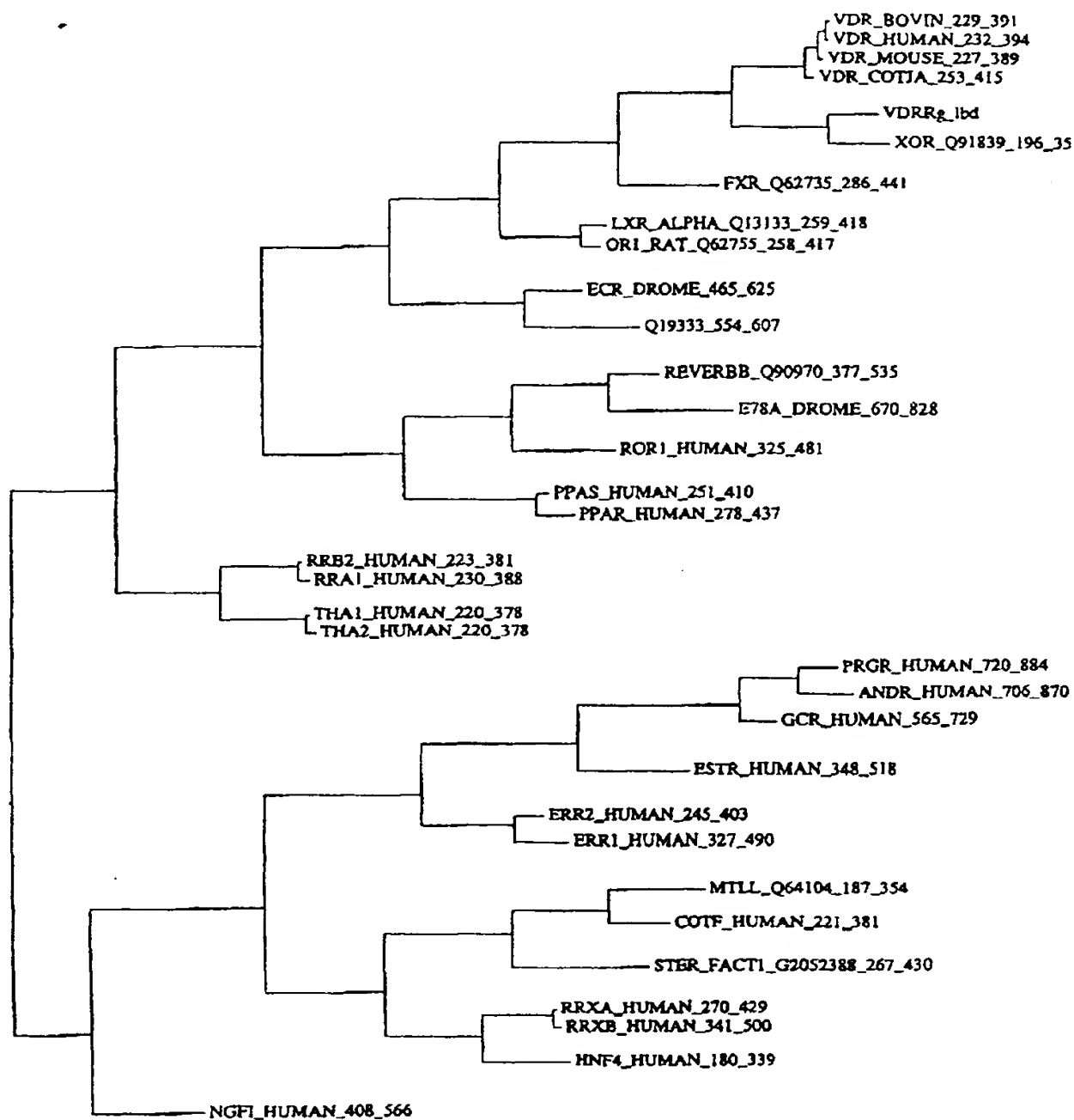


Fig. 3



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1 MEVRPKESWN HADFVHCEDT ESVP GKPSVN ADEEVGGPQI CRVCGDKATG  
51 YHFNVMTC EG CKGFFRRAMK RNARLRCPFR KGACEITRKT RRQCQACRLR  
101 KCLESGMKKE MIMSDEAVEE RRALIKRKKS ERTGTQPLGV QGLTEEQRMM  
151 IRELMDAQMK TFDTTFSHF K NFRLPGVLSS GCELPESLQA PSREEAAKWS  
201 QVRKDLCSLK VSLQLRGEDG SVWNYKPPAD SGGKEIFSL PHMADMSTYM  
251 FKGHSFAKV ISYFRDLPIE DQISLLKGAA FELCQLRFNT VFNAETGTWE  
301 CGRLSYCLED TAGGFQQLL EPMLKFHYML KKLQLHEEEY VLMQAISLFS  
351 PDRPGVLQHR VVDQLQEQA ITLKSYTECN RPQPAHRFLF LKIMAMLT EL  
401 RSINAQHTQR LLRIQDIHPF ATPLMQELFG ITGS

Fig. 4

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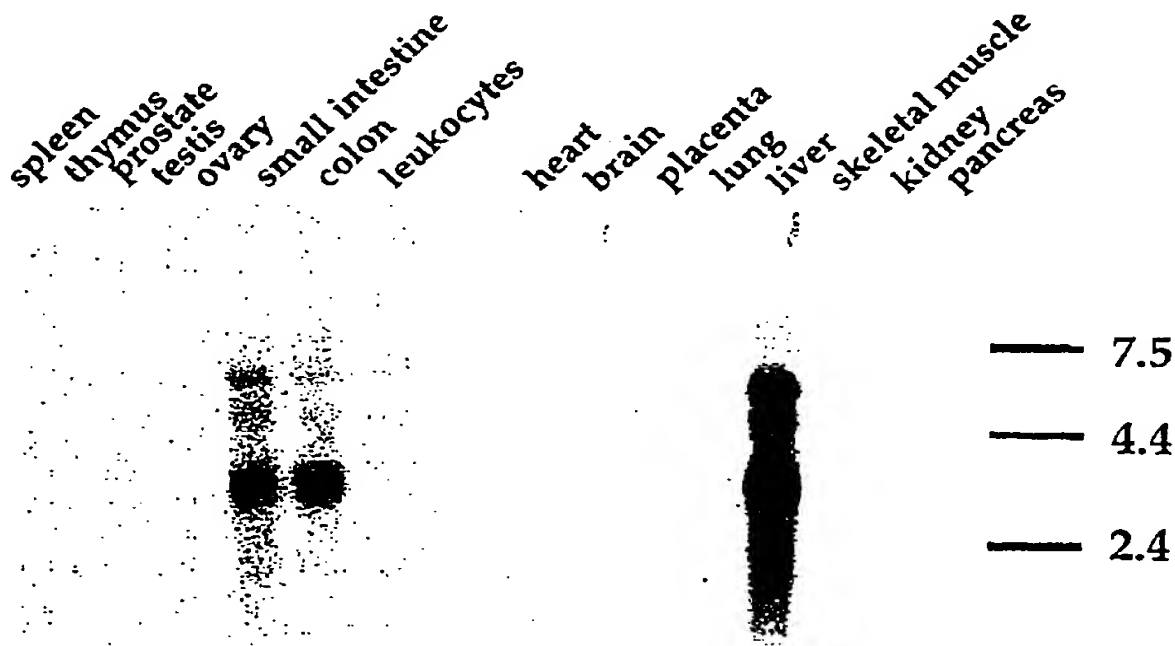


Fig. 5

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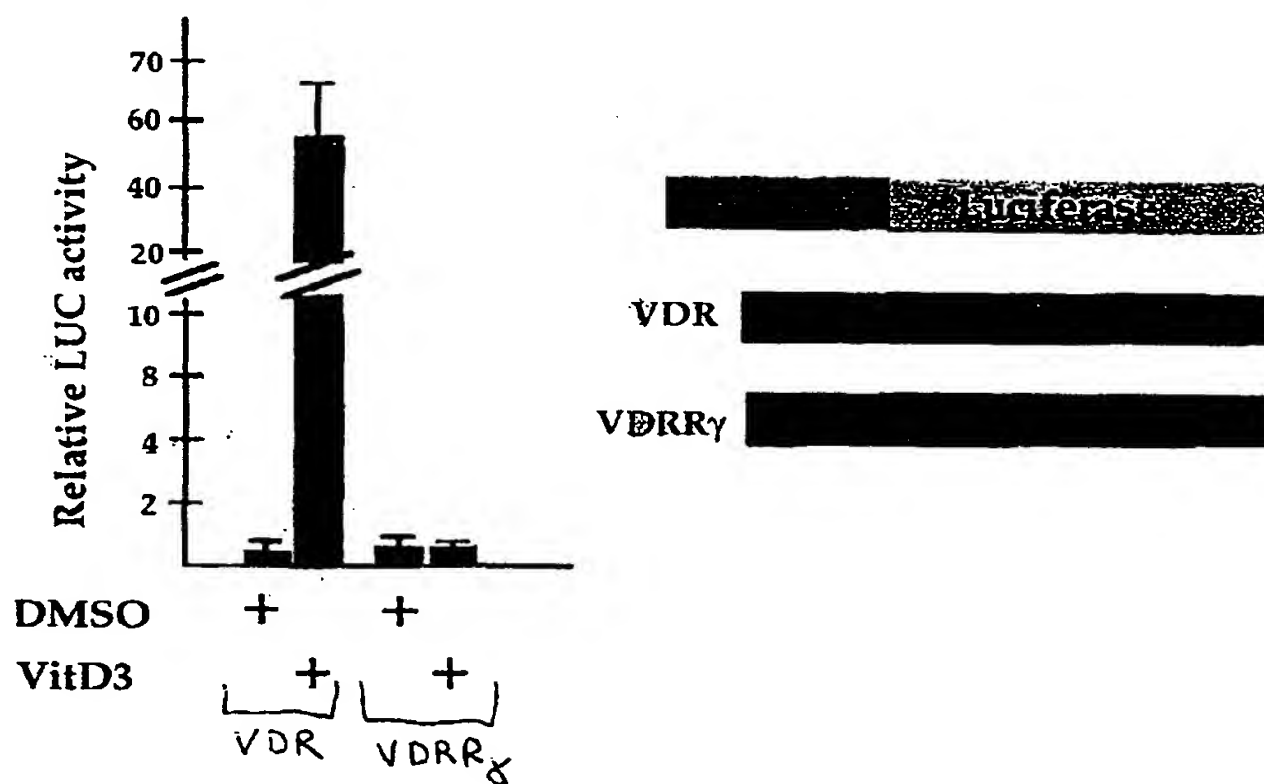


Fig. 6

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TGAATTGCTGGGCCCTGGCTGGGTTAGTGCTGGCAGCCCCC 40  
 TGAGGCCAAGGACAGCATGACAGTACCCAGGACTCAC 80  
 CACTTCAAGGAGGGTCCCTCAGAGCACCTGGCATACCCC 120  
 TGCACAGTGTCTGGGCTGAGTTGGCTTCAAACCATCCAAG 160  
 AGGCCCAGAAGCAAACCTGGAGGTGAGACCCAAAGAAAGC 200  
 TGGAAACATGCTGACTTTTGTACACTGTGAGGACACAGAGT 240  
 CTGTTCTCTGGAAAGCCCCAGTGTCAACGCAGATGAGGAAGT 280  
 CGGAGGTCCCCAAATCTGGCGTGTATGTGGGGACAAGGOC 320  
 ACTGGCTATCACTTCAATGTTCATGACATGTGAAGGATGCA 360  
 AGGGCTTTTTCAGGAGGGCCATGAAAAGCAACGCCCGGCT 400  
 GAGGTGCCCCCTTCGGGAAGGGCGGCTGGCAGATCACCCGG 440  
 AAGACCCGGGACAGTGGCCAGGOCCTGGCGCTTGGCAAGT 480  
 GCCTGGAGACCCGCATGAAGAAGGAGATGATCATGTCCGA 520  
 CGAGGCCGTGGAGGAGAGGGGGGCTTIGATCAAGCGGAAG 560  
 AAAAGTGAACCGACAGGACTCAGCCACTGGCAGTGGCAGG 600  
 GGCTGACAGAGGAGCAGCGGATGATGATCAGGGAGCTGAT 640  
 GGAGGCTCAGATGA AAAACCTTTGACACTACCTTCTCCCAT 680  
 TTCAAGAATTTCCGGCTGGCAGGGGTGCTTAGCAGTGGCT 720  
 GCGAGTTGCCAGAGTCTCTGCAGGCCCCATCGAGGGAAGA 760  
 AGCTGCCAAGTGGAGCCAGGTCCGGAAAGATCTGTGCTCT 800  
 TTGAAGGTCTCTCTGCAGCTCGGGGGGGAGGATGGCAGTG 840  
 TCTGGAACIACAAACCCCCAGCCGACAGTGGCGGGAAGA 880  
 GATCTTCTCCCTGCTGGCCCCACATGGCTGACATGTCAACC 920  
 TACATGTTCAAAGGCATCATCAGCTTTGCCAAAGTCACT 960  
 CCTACTTTCAGGACTTGGCCATGAGGACCCAGATCTCCCT 1000  
 GCTGAAGGGGGCCGCTTTCCAGGCTGTGTCAACTGAGATT 1040  
 AACACAGTGTTCACCGCGGAGACTGGAACTGGGAGTGTG 1080  
 GCGGCTGTCTACTGCTTTGGAAGACACITGCAGGTGGCTT 1120  
 CCAGCAACTTCTACTGGAGCCCATGCTGAAATTCACACTAC 1160  
 ATGCTGAAGAGCTGCAGCTGCATGAGGAGGATATGTGC 1200  
 TGATGCAGGOCATCTCCCTCTTCTCCCCAGACCGGCCAGG 1240  
 TGTGCTGCAGCAACCGGIGGTTGGAACAGCTGCAGGAGCAA 1280  
 TTUGCCATTACTCTGAAGTCCCTACATTGAATGCAATCGGC 1320  
 CCCAGCCCTGCTCATAGGTTCTTGTCTCTGAAGATCATGGC 1360  
 TATGCTCACCCAGCTCCGCAGCATCAATGCTCAGCACACC 1400  
 CAGCGGCTGCTGGCATCCAGGACATACACCCCTTTGCTA 1440

Fig. 7

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CGCCCCCTCATGCAGGAGTGTGTGCGCATCACAGGTAGCTG 1480  
 AGCGGCTGCCCTTGGGTGACACCTCCGAGAGGCAGCCAGA 1520  
 CCCAGAGCCCTCTGTAGCGGCCACTCCCGGGCCAAAGACAGA 1560  
 TGGACACTGCCAAGAGCGGACAAATGCCCTGCTGGGCTGTGTC 1600  
 TCCCATAGGGAATTCCTGCTATGACAGCTGGCTAGCATTCCT 1640  
 TCAGGAAGGACATGGGTGCCCCCACCCTCAGTTCAGTCT 1680  
 GTAGGGAGTGAAGCCACAGACTCTTACGTGGAGAGTGCAC 1720  
 TGACCTGTAGGTGAGGACCATCAGAGAGCCAAAGGTTCGCC 1760  
 TTTCTTTTAAAGGCGCTGTGGTCTGGGGAGAAATCCCT 1800  
 CAGATCCCACTAAAGTGTCAAGGTGTGGAAGGGACCAAGC 1840  
 GACCAAGGATAGGCCATCTGGGGTCTATGCCACATAACC 1880  
 ACGTTTGTTCGCTTCCTGAGTCTTTTCATGCTAOCCTCTA 1920  
 ATAGTCCCTGTCTCCCACTTCCCACTCGTTCCTCCTCTT 1960  
 CCGAGCTGCTTTGTGGGCTCAAGGCTGTACTCATCGGCA 2000  
 GGTCCATGAGTATCTGTGGGAGTCCCTCTAGAGAGATGAGA 2040  
 AGCCAGGAGGCGCTGCACCAATGTTCAGAAAGCTTGGCATGA 2080  
 CCTCATTCGGGCCACATCATTCGTGTCTCTGCTATCCATT 2120  
 TGAACACATTATTAAGCACTGATAATAGGTAGCCCTGTGT 2160  
 GGGGTATACAGCATTTGACTCAGATATAGATCCTGAGCTCA 2200  
 CAGAGTTTATAGTTTAAAAAACAACAGAAACACAAACAA 2240  
 TTTGGATCAAAAGGAGAAATGATAAGTGCACAAAGCAGC 2280  
 ACAAGGAATTTCCCTGTGTGGATGCTGAGCTGTGTATGGCA 2320  
 GGCCTGCGGTACCCAAGTGAAGGTTCCTGAGGACATGAGT 2360  
 CTGTAGGAGCAAGGGCACAACTGCAGCTGTGAGTGGGTG 2400  
 TGTGTGATTTGGTGTAGGTAGGTCTGTTCGCCACTTCATG 2440  
 GGGCTGGGTTTGTTCCTGGGGCTGGAATGCTGGGTATGC 2480  
 TCCTGTGACAAGGCTACGCTGACAATCAGTTAAACACACCG 2520  
 GAGAAGAACCATTATACATGCACCTTATATTTCTGTGTACA 2560  
 CATCTATTCTCAAGCTTAAAGGTATGAAAGTGCCTGCTCT 2600  
 TGTTTATAGCCACTTGTGTAGTAAAAATTTTTTGTGATTTT 2640  
 CACAAATTATACTTTATATAAGGCATTCCACACCTAAGAA 2680  
 CTAGTTTTTGGGAAATGTAGCCCTGGGTTTAATGTCAAATC 2720  
 AAGGCAAAAGGAATTAAATTAATGTACTTTTGGCTAAAAAA 2760  
 AA 2800  
 AA 2802

Fig. 7 (cont.)

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MTVTRTHHFKEGSLRAPAIPLHSAAAELASNHPRGPEANL 40  
 EVRPKESWNHADVFVHCEDTESVPGKPSVNADEEVGGPQIC 80  
 RVC GDKATGYHFNVMTCEGCKGFFRRAMKRNARLRCPPFRK 120  
 GACEITRKTRRQCQACRLRKCLESGMKKEMIMSDEAVEER 160  
 RALIKRKKCERTGTQPLGVQGLTEEQRMMIRELMDAQMKT 200  
 FDTTFSHFKNFRLPGVLSSGCELPESLQAPSREEAAKWSQ 240  
 VRKDLCSLKVSLQLRGEDGSVWNYKPPADSGGKEIFSLLP 280  
 HMADMSTYMFKGIISFAKVISYFRDLPIEDQISLLKGA AF 320  
 ELCQLRFNTVFNAETGTWECGRLSYCLEDTAGGFQQLLLE 360  
 PMLKFHYMLKKLQLHEEEYVLMQAI SLFSPDRPGVLQHRV 400  
 VDQLQEQFAITLKS YIECNRPQPAHRFLFLKIMAMLT ELR 440  
 SINAQHTQRLRLRIQDIHPFATPLMQELFGITGS. 474

Fig. 8

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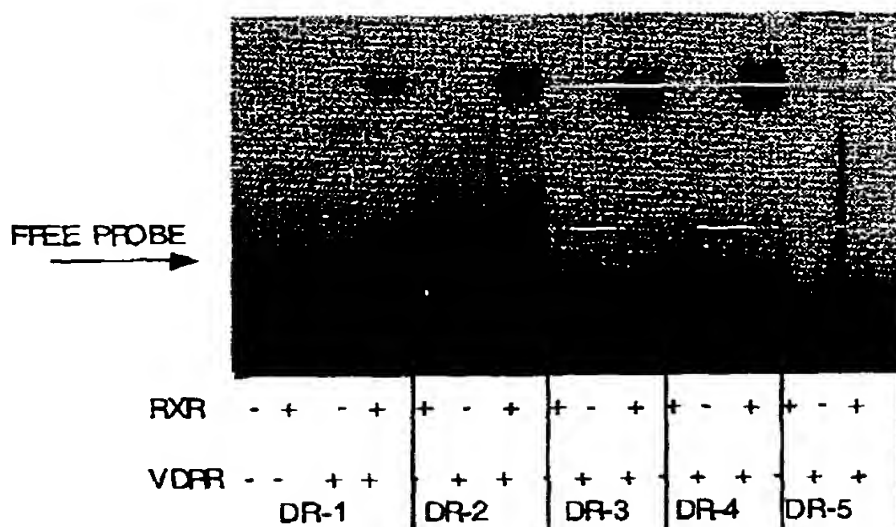


Fig. 9

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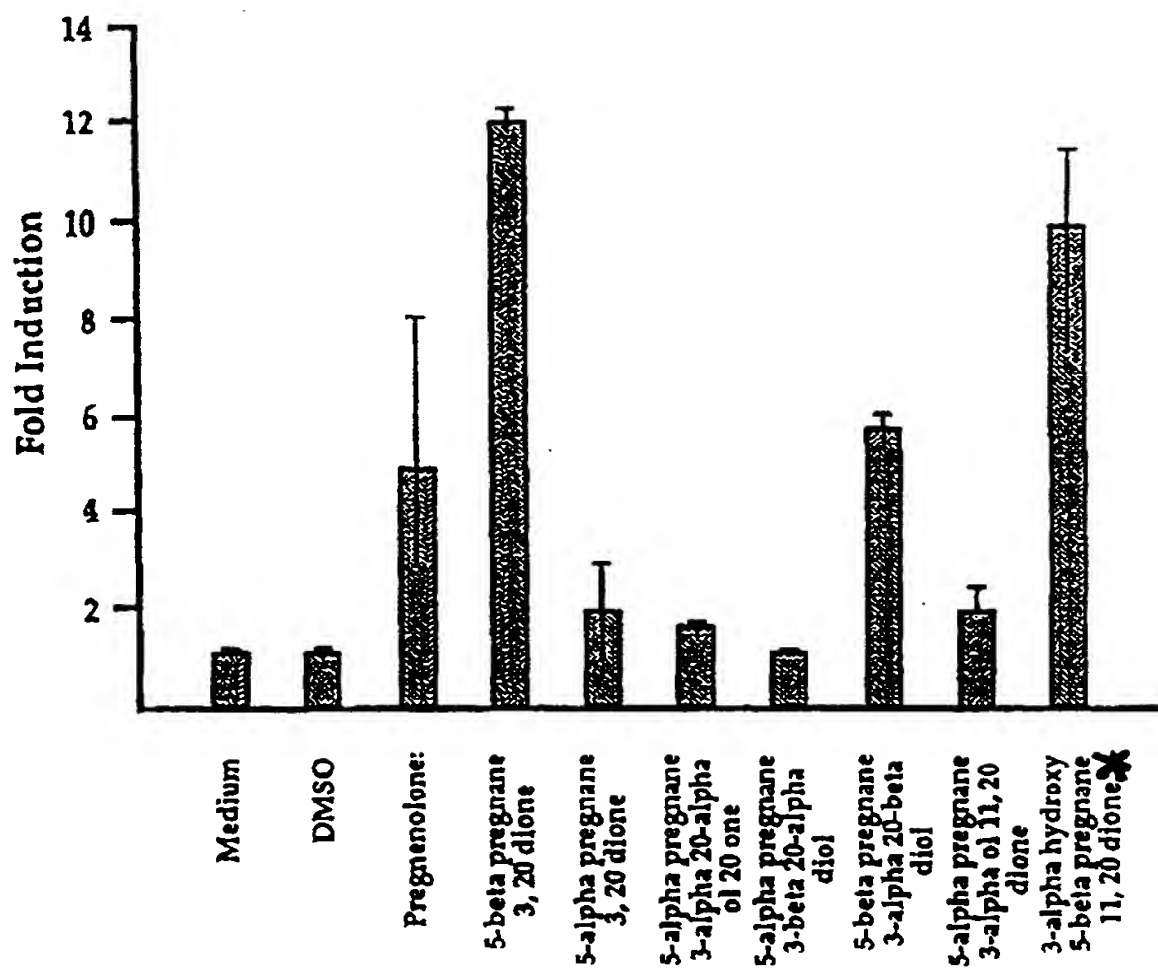


Fig. 10



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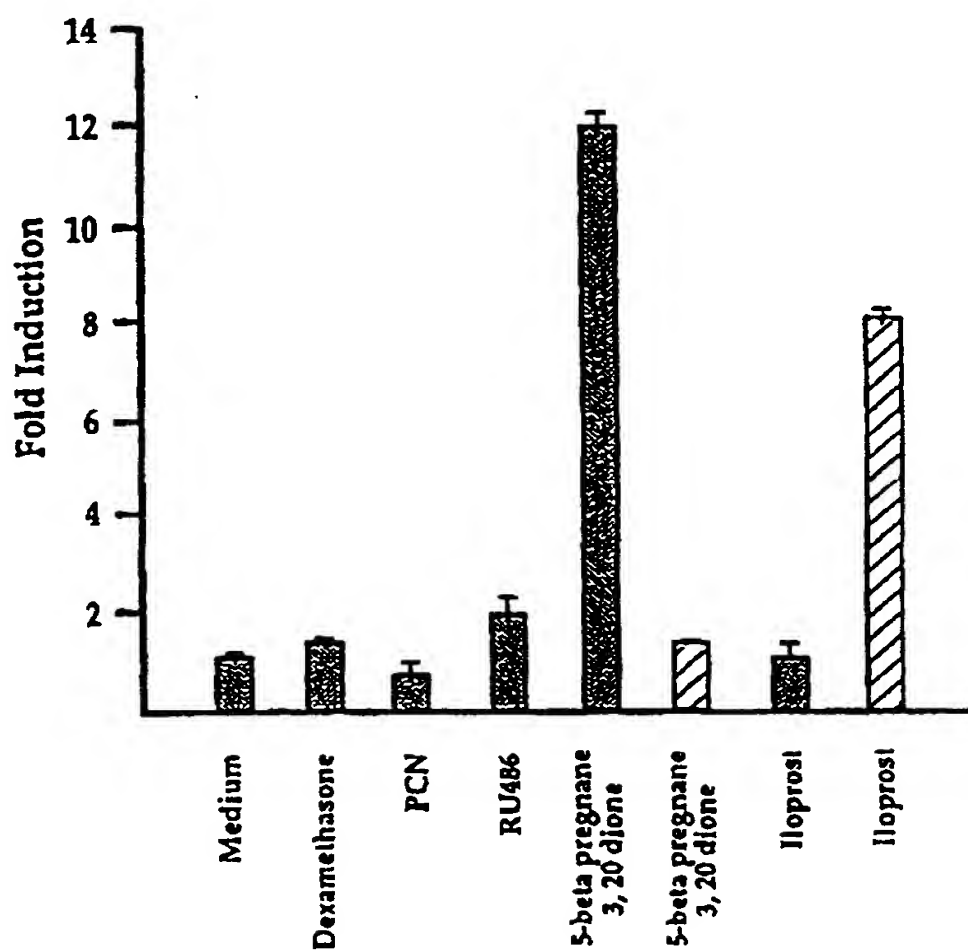


Fig. 11